Serological screening of Contagious Bovine Pleuropneumonia in goats: the specificity challenge (Abstract n°265)

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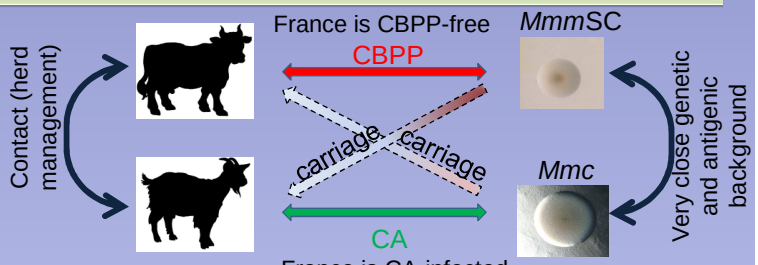
Background: Surveillance of Contagious bovine pleuropneumonia (CBPP) in a currently free country, a complex challenge.

CBPP is a severe respiratory disease of cattle and buffalo caused by *Mycoplasma mycoides* subsp. *mycoides* biotype Small Colony (*Mmm*SC). The agent of CBPP was recently isolated from goats at different times and places, even in areas free of CBPP. Thereby, goats should be considered a putative *Mmm*SC reservoir. No test has been proposed so far for surveillance of CBPP in goats. Furthermore, serological tests might be seriously hampered by a common caprine infection due to *M. mycoides* subsp. *capri* (*Mmc*), a subspecies that is antigenically very close to *Mmm*SC.



42% of mycoplasmas isolated in goats are *Mmc**

Aim: This study was conducted to assess whether the competitive ELISA currently recommended by the OIE for CBPP screening at the herd level in cattle was suitable for caprine monitoring notably in terms of specificity.

Methods and Results1: cELISA serology as a herd test

325 sera were collected from 11 goat herds (~30 sera/herd). Six herds were considered *Mmc*-infected (isolation of *Mmc* strains from bulk milk tanks) while 5 other herds were considered non-infected. Competition ELISA titers were measured as indicated by the supplier (Institut Pourquier/CIRAD). cELISA titers were comparable within the 2 populations and were altogether normally distributed with a mean of 24+/-12% (Fig.1A). The resulting bell curve was below the cut-off established for bovine sera positivity (i.e. inhibition >50%—).

In comparison, bovine sera from a randomly chosen population of 309 herds (~2 sera/herd) gave cELISA results very similar to that obtained with goats (with a mean of 29+/-10%) (Fig.1B). All bovine sera -but one doubtful- were negative, as expected.

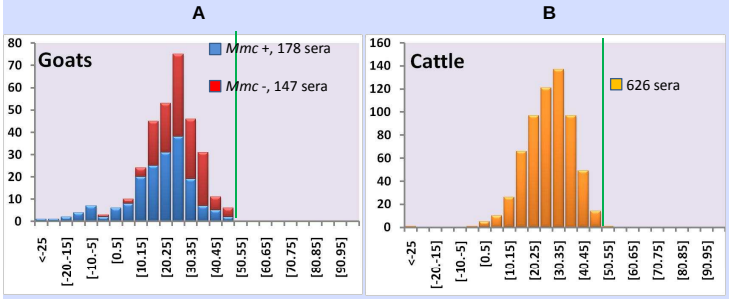


Fig.1: cELISA results (distribution of sera as a function of the % of inhibition)

Methods & Results 2: Description of a 3F3+ *Mmc* strain

Strain #14690 was isolated in 2006 in France from the joint of a goat with arthritis. It reacted with the 3F3 monoclonal antibody specific to *Mmm*SC (and targeted by the cCBPP ELISA kit) but was identified (sequence analysis) as a *Mmc*.

Such *Mmc* strains are of rare occurrence and only 2 were isolated in France between 2003 and 2008 out of 550 screened *Mmc* strains (0.4%).*

The 3F3-coding sequence (87 nt) was amplified with specific primers and compared between several strains (11 *Mmm*SC and 25 *Mmc*). The region is highly conserved within the *Mmm*SC taxon but very variable within the *Mmc* subspecies (multiple, long branches in the resulting phylogenic tree, Fig.2). Strain #14690 shows 6/87 nucleotides polymorphism when compared to the consensus sequence of *Mmm*SC resulting in only 2 amino acid differences (D<->E and N<->D).

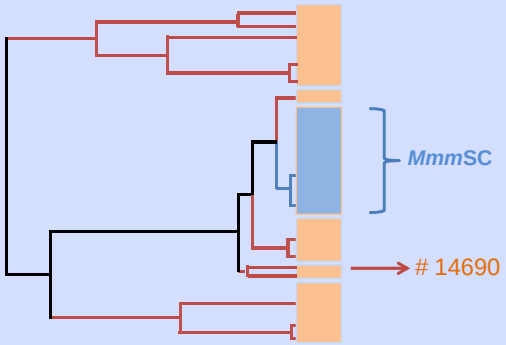


Fig.2: Phylogenic tree constructed by the Neighbour Joining method (1000 iterations) on the 87 bp 3F3 coding sequence. The *Mmc* strains are grouped under the « orange » boxes

Methods & Results 3: cELISA analysis of the experimentally produced antisera against *Mmc#14690*

Goat sera produced by subcutaneous inoculation of *Mmc* #14690 (2 ml x ~10^8 cfu/ml) were analyzed by CBPP-cELISA .

A significant seroconversion - comparable to that obtained in goats after inoculation of a *Mmm*SC pathogenic strain and above the positivity threshold for cattle - was evidenced for 2 goats (Fig.3).

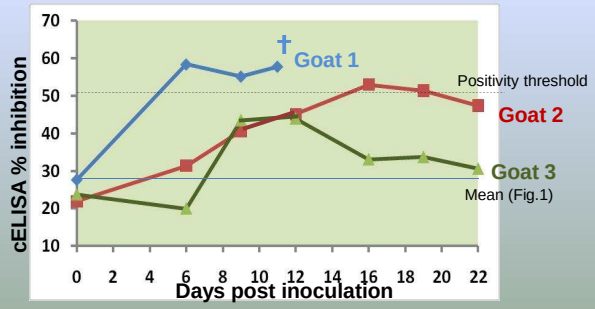


Fig.3: cELISA follow-up of goats sera after inoculation of Mmc#14690

Conclusions

The commercially available cELISA can be used in a CBPP-free context for both goat and cattle monitoring.

In goats, the test sensitivity has yet to be assessed. In general, its specificity is not hampered by circulation of *Mmc* strains and the falsely positive reactions induced by 3F3+ *Mmc* strains are poorly probable in field conditions since occurrence of 3F3+ *Mmc* strains is random (due to genetic polymorphisms) and unsual (0.4%)*.

In cattle, presence of *Mmc* strains in clinical specimens is rare (0.4%)*. *Mmc* circulation in a non-clinical context has yet to be documented but should not – by analogy with the situation in goats- influence the overall cELISA test specificity.

*** Data from the French network VIGIMYC between 2003-2008.**